The triple oxygen isotope composition of phytoliths as a proxy of continental atmospheric humidity: insights from climate chamber and climate transect calibrations

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Abstract

Continental atmospheric relative humidity (RH) is a key climate-parameter. Combined with atmospheric temperature, it allows us to estimate the concentration of atmospheric water vapor which is one of the main components of the global water cycle and the most important gas contributing to the natural greenhouse effect. However, there is a lack of proxies suitable for reconstructing, in a quantitative way, past changes of continental atmospheric humidity. This reduces the possibility to make model-data comparisons necessary for the implementation of climate models. Over the past 10 years, analytical developments have enabled a few laboratories to reach sufficient precision for measuring the triple oxygen isotopes, expressed by the $^{17}$O-excess ($^{17}$O-excess = ln ($^{17}$O / $^{18}$O) – 0.528 x ln ($^{18}$O / $^{16}$O)), in water, water vapor and minerals. The $^{17}$O-excess represents an alternative to deuterium-excess for investigating relative humidity conditions that prevail during water evaporation. Phytoliths are micrometric amorphous silica particles that form continuously in living plants. Phytolith morphological assemblages from soils and sediments are commonly used as past vegetation and hydrous stress indicators. In the present study, we examine whether changes in atmospheric RH imprint the $^{17}$O-excess of phytoliths in a measurable way and whether this imprint offers a potential for reconstructing past RH. For that purpose, we first monitored the $^{17}$O-excess evolution of soil water, grass leaf water and grass phytoliths in response to changes in RH (from 40 to 100 %) in a growth chamber experiment where transpiration reached a steady state. Decreasing RH decreases the $^{17}$O-excess of phytoliths by 4.1 per meg / % as a result of kinetic fractionation of the leaf water subject to evaporation. In order to model with accuracy the triple oxygen isotope fractionation in play in plant water and in phytoliths we recommend direct and continuous measurements of the triple isotope composition of water vapor.

Then, we measured the $^{17}$O-excess of 57 phytolith assemblages collected from top soils along a
RH and vegetation transect in inter-tropical West and Central Africa. Although scattered, the $^{17}\text{O}$ excess of phytoliths decreases with RH by 3.4 per meg / %. The similarity of the trends observed in the growth chamber and nature supports that RH is an important control of $^{17}\text{O}$-excess of phytoliths in the natural environment. However, other parameters such as changes in the triple isotope composition of the soil water or phytolith origin in the leaf tissue may come into play. Assessment of these parameters through additional growth chambers experiments and field campaigns will bring us closer to an accurate proxy of changes in relative humidity.

1 Introduction

Continental atmospheric relative humidity (RH) is a key climate-parameter. Combined with atmospheric temperature, it allows scientists to estimate the concentration of atmospheric water vapor which is one of the main components of the global water cycle and the most important gas contributing to the natural greenhouse effect (e.g. Held and Soden, 2000; Dessler and Davis, 2010; Chung et al., 2014). However, global climate models (GCMs) have difficulties to properly capture continental humidity conditions (Sherwood et al., 2010; Risi et al., 2012; Fischer and Knutti, 2013). Although tropospheric RH results from a subtle balance between different processes (including air mass origins and trajectories, large scale radiative subsidence, evaporation of falling precipitation, detrainment of convective system, evapotranspiration), it is usually depicted as rather constant in GCMs in agreement with thermodynamic coupling between atmospheric water vapor and sea surface temperature (Bony et al., 2006). A model-data comparison approach is thus essential to progress on this issue. This approach has to be applicable beyond the instrumental period to make use of past changes in atmospheric water vapor conditions.

There are multiple ways to reconstruct past continental temperature and precipitation, for instance from pollen (Bartlein et al., 2010; Herbert and Harrison, 2016; Wahl et al., 2012) or tree ring data (Labuhn et al., 2016; Lavergne et al., 2017). However, there is a serious lack of proxies suitable for reconstructing, in a quantitative way, past variations in continental atmospheric RH. Indeed, the stable isotopes of oxygen and hydrogen ($^{18}\text{O}$ and $\delta D$) of tree rings can be influenced by several parameters other than humidity (precipitation source, temperature). This limits the interpretation of tree ring isotope series in terms of humidity changes to places where variations of these other parameters are well constrained (Grießinger et al., 2016; Wernicke et al., 2015). A promising method relies on the $^{18}\text{O}$ and $\delta D$ of plant biomarkers (e.g. n-alkanes and fatty acids from leaf waxes) recovered from soils (or buried soils) and sediments. It allows for an estimate in changes in plant water deuterium-excess ($d$-excess = $\delta D$ - 8.0 x $^{18}\text{O}$), linked to changes in precipitation sources and RH. This method under development can however be biased by factors other than climatic such as plant functional types and selective degradation of the biomarkers (e.g. Schwab et al., 2015; Tuthorn et al., 2015).

Phytoliths are micrometric amorphous silica ($\text{SiO}_2$, $\text{nH}_2\text{O}$) particles that form continuously in living plants. Silicon is actively absorbed by the roots (Ma and Yamaji, 2006) and is translocated in the plant tissues where it polymerizes inside the cells, in the cell walls and in extracellular spaces.
of stems and leaves. Silica polymerization appears to be an active physiological process, which does not only depend on transpiration (Kumar et al., 2017). In grasses, which are well known silica accumulators, silica accounts for several % of dry weight (d.w.) and is mainly located in the stem and leaf epidermis. Phytolith morphological assemblages from soils and sediments are commonly used as past vegetation and hydrolytic stress indicators (e.g. Aleman et al., 2012; Backwell et al., 2014; Bremond et al., 2005a, 2005b; Contreras et al., 2014; Nogué et al., 2017; Piperno, 2006). The potential of the δ18O signature of phytoliths (δ18Ophylo) from grasses for paleoclimate reconstruction has been investigated through growth chamber and North American Great Plains calibrations. It has been shown that the δ18Ophylo of grass stems weakly affected by transpiration correlated with the δ18O signature of soil water (δ18Osw) and the atmospheric temperature, as expected for a polymerization of silica in isotope equilibrium with the plant water (Webb and Longstaffe, 2000, 2002, 2003, 2006). This was not the case for δ18Ophylo from grass leaves that correlated with RH as expected for an evaporative kinetic isotope enrichment of the leaf water (e.g. Cernusak et al., 2016) imprinted on δ18Ophylo. However, because grass stem and leaf phytoliths have the same morphology and are mixed in soil and sedimentary samples, these calibrations were not sufficient for using δ18Ophylo of grassland phytolith assemblages as a paleoclimatic signal. In tropical trees, silica is found in leaves, bark and wood and accounts for a few % d.w. (e.g. Collura and Neumann, 2017). In the wood, silica polymerizes in the secondary xylem supposedly unaffected by transpiration, in the form of Globular granulate phytolith types (Madella et al., 2005; Scurfield et al., 1974; Welle, 1976). These phytoliths make up more than 80% of tropical humid forest and rainforest phytolith assemblages found in soils and sediments (Alexandre et al., 2013; Collura and Neumann, 2017; Scurfield et al., 1974; Welle, 1976). Examination of the δ18Ophylo of rainforest assemblages showed correlations with the δ18O of precipitation (δ18Opre) and the atmospheric temperature (Alexandre et al., 2012). However, in this case, the use of δ18Ophylo did not further develop because it was applicable only to forested areas and humid climatic periods, which is a major drawback for paleoclimatic reconstructions.

The triple isotope composition of oxygen in the water molecule represents an alternative for investigating RH conditions prevailing during water evaporation. In the triple isotope system, the mass-dependent fractionation factors between A and B (17O/A-B and 18O/A-B) are related by the exponent \( \theta_{A-B} = \ln(A^{17}B / A^{18}B) \) or \( \theta_{A-B} = \ln(17O/A-B) / \ln(18O/A-B) \). In delta notation, the relationship becomes \( \theta_{A-B} = \ln(1 + D_{A-B}) / \ln(1 + D_{A-B}) \), where \( A^{*}_{A-B} = (\delta_{A} - \delta_{B}) / (\delta_{A} + 1) \) is expressed in % e. * denotes 17 or 18. This expression of \( A^{*}_{A-B} \) is more accurate than its linearized form \( \Delta_{A-B}^{*} = \Delta_{A-B}^{*} - \delta_{B}^{*} \) (Angert et al., 2003; Miller, 2002). It has been recently empirically estimated that \( \theta \) equals 0.529 for liquid-vapor equilibrium (\( \theta_{eq} \); Barkan and Luz, 2005) and 0.518 for vapor diffusion in air (Barkan and Luz, 2007). The triple oxygen isotope composition can also be described graphically in a ln (δ17O + 1) vs ln (δ18O + 1) space, in which \( \theta \) represents the slope of the data alignment during a mass-dependent fractionation process. The ln expression of δ17O and δ18O are referred to as δ17O and δ18O. In this space, meteoric waters plot along a line with a slope of \( \theta \).
0.528 ± 0.001. The departure from the meteoric water line is conventionally called \(^{17}\text{O}\)-excess (\(^{17}\text{O}\)-excess = \(\delta^{17}\text{O} \times 0.528 \times \delta^{18}\text{O}\)) (Luz and Barkan, 2010). In case of mass-dependent fractionation processes, the magnitudes of the \(^{17}\text{O}\)-excess in waters and minerals are very small and measurement of the \(^{17}\text{O}\)-excess, expressed in per meg (10\(^{-3}\)%o) vs VSMOW, requires to reach very high analytical precisions.

In the water cycle, the \(^{17}\text{O}\)-excess variations mainly result from diffusion processes, while equilibrium fractionation does not lead to important departure from the meteoric water line. Theoretical and empirical estimations have shown that in contrast to d-excess, and except at very high latitudes, changes in water \(^{17}\text{O}\)-excess are not significantly impacted by temperature (~0.1 per meg / °C; Uemura et al., 2010) and much less sensitive to distillation processes (Angert et al., 2004; Barkan and Luz, 2007; Landais et al., 2008; Uemura et al., 2010; Steig et al., 2014). Changes in water \(^{17}\text{O}\)-excess are thus essentially controlled by evaporative kinetic fractionation. The \(^{17}\text{O}\)-excess decreases in the evaporating water and increases in the vapor phase when RH decreases at evaporative sites (e.g. sea surface, lake surface, soil surface or leaf surface). Over the last ten years, a few studies used the \(^{17}\text{O}\)-excess of water to interpret ice core archives in climatic terms (Guillevic et al., 2014, Schoeneman et al., 2014; Winkler et al., 2012; Landais et al., 2008, 2012).

They supported that \(^{17}\text{O}\)-excess is a marker of RH, sea-ice extent at the moisture source, and air mass mixing (Risi et al., 2010) except at the very high latitudes of East Antarctica where temperature can have a significant influence. The observed variations of \(^{17}\text{O}\)-excess in Greenland ice cores of ~20 per meg maximum were thus interpreted as variations of RH or sea-ice extent at the source region and coincide with variations in the low to mid latitude water cycle as recorded by other proxies (such as CH\(_4\) or \(\delta\text{D}\) of CH\(_4\)) (Guillevic et al., 2014). An even smaller number of studies measured or attempted to model the \(^{17}\text{O}\)-excess of rainwater at low and temperate latitudes (Affolter et al., 2015; Landais et al., 2010b; Li et al., 2015; Luz and Barkan, 2010; Risi et al., 2013). The observed variations in \(^{17}\text{O}\)-excess, partly explained by convective processes and re-evaporation of precipitation, were of the order of 30-40 per meg, either during a rainy event or along climatic gradients. Only two studies focused on open surface waters, and showed that variations of the \(^{17}\text{O}\)-excess ranged from tens to hundreds of per meg when the surface water underwent strong evaporative enrichment (Surma et al., 2015; Luz and Barkan, 2010), in agreement with the Craig and Gordon (1965) formulation. The most important variations in \(^{17}\text{O}\)-excess occur at the plant-atmosphere interface. In leaf water, variations higher than 200 per meg were encountered (Landais et al., 2006; Li et al., 2017). Difference in \(^{17}\text{O}\)-excess between leaf water subject to evaporation (LW) and stem water (SW) not subject to evaporation, increased with decreasing RH (from 100 to 30 %), as expected for processes dominated by kinetic fractionation. When measuring a sequence of LW - SW couples sampled under different climatic conditions, the slope of the line linking their triple isotope composition and named \(\lambda_{\text{LW-SW}}\), equivalent to \(\theta_{\text{LW-SW}}\), was found to change with RH. This pattern was neither influenced by the plant species nor by the environmental conditions (e.g. atmospheric temperature, soil water conditions) (Landais et al., 2006). However opposite trends of \(\lambda_{\text{LW-SW}}\) with RH were observed from one study to another.
(Landais et al., 2006; Li et al., 2017). This discrepancy was attributed to the possibility that steady state is not always reached during sampling and to likely differences in isotope composition of the ambient vapor, a parameter of the Craig and Gordon model that is often not measured but estimated (Li et al., 2017).

While $^{17}$O-excess measurements of waters were expanding, analyses of the triple oxygen isotope composition of minerals (mostly silicates and carbonates) were also developed, allowing estimate of fractionation during polymerization and providing constraints on both temperature and isotope composition of the water source (Pack and Herwartz, 2014; Levin et al., 2014; Passey et al., 2014; Herwartz et al., 2015; Miller et al., 2015; Sharp et al., 2016). Variations of $^{17}$O-excess of the order of tens to hundreds of per meg were reported from one mineral to another. For most of the studies cited above, the objective was to discriminate between high and low temperature processes or to decipher from which type of water the mineral formed (i.e. sea water, hydrothermal water, meteoric or surface water). The $^{17}$O-excess of biogenic and sedimentary carbonates was also investigated as a potential record of evaporating water sources (Passey et al., 2014). With regard to silicate-water fractionation, the relationship between the three oxygen isotopes defined by $\theta_{\text{SiO}_2-\text{water}}$ was estimated between 0.521 and 0.528, increasing logarithmically with temperature (Sharp et al., 2016).

In the present study, in the light of the recent findings cited above, we examined whether changes in atmospheric RH imprint the $^{17}$O-excess of phytoliths ($^{17}$O-excess$^{\text{phyto}}$) in a measurable way and whether this imprint offers a potential for reconstructing past RH. For that purpose, we first monitored the $^{17}$O-excess evolution of soil water, grass leaf water and grass phytoliths in response to changes in RH in a growth chamber experiment. Then, we measured the $^{17}$O-excess$^{\text{phyto}}$ from 57 phytolith assemblages collected in soil tops along a RH and vegetation transect in inter-tropical West and Central Africa. Relationships between $^{17}$O-excess$^{\text{phyto}}$ and RH were looked for and assessed on the basis of previous quantifications of kinetic isotope enrichment of leaf water and equilibrium fractionation between water and silica. Results from the natural sampling were compared to the ones from the growth chamber experiment to evaluate the importance of RH in controlling $^{17}$O-excess$^{\text{phyto}}$ in natural environment.

2 Materials and methods

2.1 Samples from the growth chamber experiment

*Festuca arundinacea*, commonly referred to as tall fescue, is widely distributed globally as a forage and an invasive grass species (Gibson and Newman, 2001) and can adapt to a wide range of conditions. In 2016, *F. arundinacea* (Callina RAGT Semences) was grown in three chambers under three conditions of RH (ca. 40, 60 and 80 %) kept constant using wet air introduction and ultrasonic humidifier. We checked that the humidifiers did not lead to any isotope fractionation between the water in their reservoirs and the vapor delivered. Temperature and light intensity were kept constant at $25 \pm 0.6$ (SD) °C and $293 \pm 14$ (SD) mmol / m$^2$ / sec respectively.

In a 35 L tank (53 x 35 x 22 cm), 20 kg of dried commercial potting soil were packed above a 1.6
cm layer of quartz gravel. A porous cup for water extraction was placed in the soil with its extraction tube hermetically extending outside of the tank walls. The soil was irrigated with 10 L of the same water as the one used for the humidifier. Four grams of seeds were sown along four rows in each tank, resulting in about 6000 seedlings. Each tank was then placed in a chamber and was irrigated from a Mariotte bottle (25 L) placed next to it. The Mariotte system was set so that a saturated level of 5 cm remained constant at the base of the tank. The irrigation water was supplemented with 105 mg/L of SiO$_2$ (in the form of SiO$_2$ K$_2$O). Ten days after germination, agar-agar (polysaccharide agarose) was spread on the soil surface around the seedlings (about 8 cm tall), to prevent any evaporation (Alexandre et al., 2016).

A fourth tank was kept at 100% of RH thanks to the installation of a 20 cm high plexiglass cover, in a forth chamber set at 80 % of RH. In this case no agar-agar was added and the vapor around F. arundinacea came from evaporation and transpiration of the soil water. Otherwise the treatment was the same as in the other chambers.

For each humidity condition, three to four harvests were made at intervals of 10-14 days. The 20-25 cm long leaves were cut at two cm above the soil level and weighed. From the first to the fourth sampling, the harvested wet leaves increased from 15-20 g (10 days of growth) to 40-60 g (14 days of growth). Three to five g of leaves were put in glass gastight vials and kept frozen for bulk leaf water extraction. The remaining leaves were dried for phytolith extraction. Forty mL of irrigation water from the Mariotte bottle, and of soil water from the porous cup, were kept at 5°C before analyses.

After each harvest, the tanks were left in their chamber of origin but the 40, 60 and 80 % RH treatments were rotated between the growth chambers so that the four replicates of a given RH treatment would come from at least two different chambers. The 100 % humidity was set up in a unique chamber during the entire duration of the experiment. The harvested leaves in this treatment were often covered by condensation drops which were blotted between two sheets of wiping paper, rapidly after harvesting. The experimental setup details and the harvest list are given in table 1.

### 2.2 Samples from the natural climate transects

Fifty-seven top soil samples were collected during several field trips along vegetation and humidity transects in Maurtania and Senegal (Bremond et al., 2005b; Lézine, 1988; Pasturel, 2015) (Lezine, 1988) Gabon (Lebamba et al., 2009) and Congo (Alexandre et al., 1997) in the saharian, sahelian, sudanian, guinean and congolian bioclimatic zones, respectively (White et al., 1983). Samplings, phytolith extractions and phytolith morphological assemblages descriptions are given in the above-mentioned studies, except for the samples of Gabon from which phytoliths were chemically treated and counted in the frame of the present study.

The sampled site location as well as the associated climatic and oxygen isotope variables are given in Table 2. The vegetation overlying the sampled soils was categorized into savanna (Maurtania, Senegal), wooded savanna (Senegal), humid forest (Gabon and Congo) and enclosed savanna (Gabon). For each sampled site, yearly climate average were calculated from the monthly means.
of temperature, precipitation, RH and diurnal temperature, extracted from the Climate Research Unit (CRU) 1961 - 1990 time series (10’ spatial resolution; http://www.cru.uea.ac.uk, Harris et al., 2013, CRU 2.0). Mean Annual Precipitation (MAP), Mean Annual Temperature (MAT) and mean annual RH range from 49 to 2148 mm, 24.3 to 29.8 ºC and 40.2 to 82.5 %, respectively. In addition, in order to get a proxy of RH during the grass growing season, averaged RH monthly means for months with at least one day with precipitation higher than 0.1 mm (RH-rd0>1) was calculated. It ranges from 56.3 to 82.5 %. As maximum transpiration is supposed to be reached around 15:00 UTC we also calculated RH and RH-rd0>1 at 15:00 (RH15 and RH15-rd0>1, respectively) according to New et al. (2002) and Kriticos et al. (2012). For each sampling site, estimates of $\delta^{18}$O of precipitation for the months with at least one day with precipitation higher than 0.1 mm ($\delta^{18}$Opre rd0>1) were extracted from The Online Isotopes in Precipitation Calculator-version OIPC2-2 (http://www.waterisotopes.org; Bowen and Revenaugh, 2003; Bowen and Wilkinson, 2002; Bowen et al., 2005) and weighted by the amount of precipitation. The estimates range from -3.22 to -4.33 ‰. There is currently no data on the $^{17}$O-excess of precipitation ($^{17}$O-excesspre) at these sites.

### 2.3 Phytolith chemical extractions

Phytoliths from soils were extracted following Crespin et al. (2008) using HCl, H$_2$O$_2$, C$_6$H$_5$Na$_3$O$_7$ and Na$_2$O$_7$S$_2$H$_2$O at 70 ºC, and a ZnBr$_2$ heavy liquid separation. It has been shown that up to a temperature of 70 ºC the extraction has no effect on the $\delta^{18}$O of phytoliths (Crespin et al., 2008). We verified that it did not have any effect on the $^{17}$O-excess either. Phytoliths from Festuca arundinaceae were extracted using a high purity protocol with HCl, H$_2$SO$_4$, H$_2$O$_2$, HNO$_3$, KClO$_3$ and KOH at 70 ºC following Corbineau et al. (2013).

### 2.4 Phytolith counting

Phytolith assemblages from the humidity transects were mounted on microscope slides in Canada Balsam, for counting, at a 600X magnification. More than 200 identifiable phytoliths with a diameter greater than 5 μm and with a taxonomic significance were counted per sample. Three repeated counting gave an error of ± 3.5 % (SD). Phytoliths were named using the International Code for Phytolith Nomenclature 1.0 (Madella et al., 2005) and categorized as Globular granulate type produced by the wood (Scurfield et al., 1974; Kondo et al., 1994), palm Globular echinate type and grass types comprising Acicular, Bulliform, Elongate psilate, Elongate echinate, Bulliform cells, and Grass Short Cells types. For each sample from the natural transects, the phytolith index d/p, a proxy of tree cover density (Alexandre and Bremond, 2009; Bremond et al., 2005a), was calculated. It is the ratio of Globular granular phytolith category (Madella et al., 2005) formed in the secondary xylem of the dicotyledon (d) wood to the grass short cell phytolith category formed in the epidermis of grasses or Poideae (p) (Collura and Neumann, 2017; Scurfield et al., 1974; Welle, 1976). Those two categories make up most of the phytolith assemblages recovered from inter-tropical soils (Bremond et al., 2005a, 2005b; Alexandre et al., 1997, 2013; Bremond et al., 2005b, 2005a).
Phytolith assemblages from the *F. arundinacea* samples were also mounted and counted. The phytolith types were categorized according to their cell of origin in the epidermis into Epidermal short cell, Epidermal long cell, Bulliform cell and Hair acicular.

### 2.5 Leaf and soil water extraction

Leaf water was extracted using a distillation line. Leaves were introduced in a glass tube connected to the distillation line, and frozen through immersion of the glass tube in liquid nitrogen. While keeping the sample frozen, the distillation line was pumped to reach a vacuum higher than $5.10^{-2}$ mbar. The pumping system was then isolated and the glass sample tube warmed to 80°C. Meanwhile, at the other end of the distillation line, a glass collecting tube was immersed in liquid nitrogen to trap the extracted water. To avoid condensation, the line between the sample tube and the collection tube was heated with a heating wire. The distillation was completed after six hours. In order to remove volatile samples from the extracted water, a few granules of activated charcoal were added and the water slowly stirred for 12 h.

Soil water was extracted using a 31mm porous ceramic cup. Brown or yellow-colored samples were filtered at 0.22µm, but remain colored after filtration, indicating the presence of soluble compounds.

### 2.6 Isotope analyses

The oxygen isotope results are expressed in the standard δ-notation relative to VSMOW.

#### 2.6.1 Phytoliths

Phytolith samples of 1.6 mg were dehydrated under a flow of N2 (Chapligin et al., 2010) and oxygen extraction was performed using the IR Laser-Heating Fluorination Technique at CEREGE (Aix-en-Provence, France) (Alexandre et al., 2006, Crespin et al., 2008; Suavet et al., 2010). The purified oxygen gas (O2) was passed through a $-114$°C slush to refreeze potential gases interfering with the mass 33 (e.g. NF) before being sent to the dual-inlet mass spectrometer (ThermoQuest Finnigan Delta Plus). The composition of the reference gas was determined through the analyses of NBS28 for which isotope composition has been set to $\delta^{18}O=9.600 \pm 0.00$, $\delta^{17}O=4.992 \pm 0.00$ and $^{17}$O-excess = -65 per meg. During the measurement period, reproducibility (SD) of the analyses of the working quartz standard (Boulangé 2008) against which the isotope composition of the sample gas was corrected on a daily basis (3 quartz standards were analysed per day) was $\pm 0.196 \pm 0.106$ %o and $\pm 22$ per meg for $\delta^{18}O$, $\delta^{17}O$ and $^{17}$O-excess respectively (n = 63; one run of eight dual inlet measurements). For every session of measurement, the effectiveness of the entire dehydration and IR-Laser-Fluorination-IRMS procedure was checked through the analysis of a working phytolith standard (MSG60) with $\delta^{18}O = 36.904 \pm 0.781$ %o, $\delta^{17}O = 19.100 \pm 0.405$ %o and $^{17}$O-excess = -215 ± 34 per meg (n = 29). For comparison, the inter-laboratory pooled value for MSG60 is $\delta^{18}O = 37.0 \pm 0.8$ %o (Chapligin et al., 2011). Recent measurements of the silicate reference materials UWG-2 garnet (Valley et al., 1995) and San Carlos (SC) olivine gave the following values: $\delta^{18}O_{UWG-2} = 5.724 \pm 0.124$ %o, $\delta^{17}O_{UWG-2} = 2.950 \pm 0.057$ %o, $^{17}$O-excess$_{UWG-2} = -68 \pm 27$ per meg (n = 5), $\delta^{18}O_{SC} = 4.949 \pm 0.219$ %o, $\delta^{17}O_{SC} = 2.561 \pm 0.122$ %o, $^{17}$O-excess$_{SC} = -49 \pm 24$ per meg (n = 5).
For comparison, silicate analyses presented in Sharp et al. (2016) are normalized to a $\delta^{18}O$ value for San Carlos Olivine of 5.3 ‰ and a $^{17}O$-excess value of -54 per meq.

### 2.6.2 Leaf water

Leaf water was analyzed at LSCE (Gif sur Yvette, France) following the procedure previously detailed in Landais et al. (2006). In summary, a fluorination line was used to convert water to oxygen using CoF$_3$ heated at 370°C in a helium flow. The oxygen was then trapped in a tube immersed in liquid helium before being analyzed by dual inlet IRMS (ThermoQuest Finnigan Delta V mass spectrometer) against a reference oxygen gas. All measurements were run against a working O$_2$ standard calibrated against VSMOW. The resulting precisions (2 runs of 16 dual inlet measurements) were 0.02 ‰ for both $\delta^{17}O$ and $\delta^{18}O$ and 5 ppm for $^{17}O$-excess.

### 2.6.3 Irrigation and soil waters

Irrigation and soil water were analyzed at the Ecotron of Montpellier (France) with an isotope laser analyzer (Picarro L2140i) operated in $^{17}O$-excess mode using an auto-sampler and a high precision vaporizer. Each water sample was used to fill three vials randomly dispatched in four groups of six samples (three replicates per sample). Each sample group was bracketed by three working standards (Giens-1, Iceberg-1 and Eco-1). Ten injections were performed for each vial, and the results of the first six injections were discarded to account for memory effects. Following IAEA recommendations (IAEA, 2013), each liquid measurement sequence was started with two vials of deionized water for instrument conditioning. The isotope compositions of each sample group were calibrated using the three interpolated mean values obtained for the bracketing working standards (Delattre et al., 2015). All isotope ratios were normalized on the VSMOW2/SLAP2 scale, with an assigned SLAP2 $^{17}O$-excess value of zero, following the recommendations of Schoenemann et al. (2013). The resulting precisions (3 replicates) were 0.018 ‰, 0.015 ‰ and 10 per meq for $\delta^{17}O$, $\delta^{18}O$ and $^{17}O$-excess ($n=31$).

The three working standards were also analyzed using the fluorination/IRMS technique used for leaf water analyses at LSCE. The $^{17}O$-excess maximum difference was 6.4 per meq, which is lower than the analytical precision obtained using the laser spectrometer.

In order to assess that soluble organic compounds present in some soil water samples did not impact the laser analyzer isotope measurements (Martín-Gómez et al., 2015), a representative set of colored samples were analyzed with and without a Picarro micro combustion module (MCM) set up between the high precision vaporizer and the analyzer inlet. This system was designed to partly remove organic volatile compounds using a catalytic process. The obtained isotope compositions were not significantly different, suggesting that organic compounds were either in low concentration, and/or did not interfere in the spectral window used by the analyzer. Therefore, the other soil water samples were analyzed without the MCM.

### 3 Results

#### 3.1 Growth chamber experiment
δ¹⁸O and ¹⁷O-excess of the irrigation water (respectively δ¹⁸O_IW and ¹⁷O-excess_IW) average -5.586 ± 0.006‰ and 26 ± 5 per meg, respectively. δ¹⁸O and ¹⁷O-excess of the soil water (respectively δ¹⁸O_SW and ¹⁷O-excess_SW) average -2.889 ± 0.188 ‰ and 16 ± 8 per meg, respectively (table S1). The isotope difference is thus significant for δ¹⁸O, less significant for ¹⁷O-excess, according to the analytical error. Although evaporative kinetic fractionation of the top soil water suctioned by the porous cup under vacuum cannot be ruled out, isotopic exchanges between the soil water and oxygen-bearing phases of the rhizosphere may also have impacted the soil water isotopic composition (Bowling et al., 2017; Chen et al., 2016; Oerter et al., 2014; Orlowski et al., 2016). Hereinafter, we consider the isotope signatures of the water absorbed by the roots of _F. arundinacea_ to be equivalent to the irrigation water that fed the saturation level at the base of the tank. This water was reached by the deepest roots, as observed on a cross-section of the soil after the end of the experiment and likely reached the upper roots by capillarity.

The transpiration of _F. arundinacea_ increases linearly from 0.03 to 0.6 L / day from 100 to 60 % RH and stabilizes around 0.6 L / day from 60 to 40 % RH (averages of the replicates, Table 1). In response to decreasing RH, δ¹⁸O (table S1) and ¹⁷O-excess (fig. 1a) values of the bulk leaf water (δ¹⁸O_LW and ¹⁷O-excess_LW) show clear increasing and decreasing trends, respectively. The averaged ¹⁸O-enrichment of bulk leaf water relatively to irrigation water (Δ¹⁸O_LW_IW) increases from 11.263 ± 4.987 and 7.516 ± 0.708 ‰ at 100 and 80 % of RH, to 14.781 ± 2.501 and then to 15.369 ± 1.829 ‰ at 60 and 40 % RH, respectively (fig. 1b; Table 1). For 100 % RH, the high standard deviations (SD) associated with δ¹⁸O_LW (table S1), and consequently with Δ¹⁸O_LW_IW (Table 1), are due to the very high δ¹⁸O_LW value of sample P3-100-10-05-16. However, as we do not have any explanation for this high value, this data was not withdrawn from the dataset. The ¹⁷O-excess values associated with the enrichment Δ¹⁸O_LW_IW (or ¹⁷O-excess_LW_IW = ln(Δ¹⁸O_LW_IW + 1) - 0.528 x ln(Δ¹⁸O_LW_IW + 1)) are scattered for a given RH. The averaged value however follows a clear pattern (fig. 1c; table 1): it is relatively similar at 100 and 80 % RH (-88 ± 48 and -75 ± 20 per meg, respectively) and decreases to -132 ± 41 and then to -159 ± 9 per meg, at 60 and 40 % of RH, respectively. The relationship between ¹⁷O-excess_LW_IW and RH from 40 to 80 % (fig.1c) can be expressed as follows:

\[ ¹⁷O-excess_LW_IW = 2.3 \times RH - 258 \]  

where ¹⁷O-excess_LW_IW is expressed in per meg vs. VSMOW and RH in %. R² is 0.72 and p < 0.0001 for the 95 % confidence interval. θ_LW_IW was calculated using the ln expression of Δ¹⁸O_LW_IW and Δ¹⁸O_LW_IW. The raw values of θ_LW_IW do not show any significant trend with RH and average 0.519 ± 0.002 (table 1).

The average phytolith content ranges from 1.1 to 0.1% d.w. Silicification of the leaf blade of _F. arundinacea_ increases with increasing transpiration and decreasing humidity (Table 1). Phytolith morphological identification shows that they formed preferentially in the epidermal short cell and to a smaller extent in the epidermal long cells (fig. 2). The proportion of silicified long cells, increases with increasing transpiration and decreasing RH (Table 1). Some hair and bulliform cells were also silicified, but in much smaller quantities. δ¹⁸O and ¹⁷O-excess of phytoliths (δ¹⁸O_Phyto
and $^{17}$O-excess$_{\text{Phyto}}$ respectively) show the same general trends with RH as $\delta^{18}$O$_{\text{LW}}$ and $^{17}$O-excess$_{\text{LW}}$ (fig. 1a, table S1).

The average value of the $^{18}$O-enrichment of phytoliths relative to the bulk leaf water ($\Delta^{18}$O$_{\text{Phyto-LW}}$) increases when RH decreases from 27.948 ± 7.168 and 28.422 ± 0.402‰ at 100 and 80 % of RH respectively to 29.335 ± 0.020 % at 60 % of RH and then 32.259 ± 2.192 % at 40% of RH (fig. 1b, Table 1). With regard to the enrichment of phytoliths relative to the irrigation water, $\Delta^{18}$O$_{\text{Phyto-IW}}$ shows the same feature as $\Delta^{18}$O$_{\text{LW-IW}}$, stabilizing from 100 to 80 % RH (36.167 ± 0.701 and 36.464 ± 0.461 %, respectively) and increasing from 80 to 40 %, to reach 48.695 ± 2.280‰ at 40 % RH (fig. 1b, table 1). $^{17}$O-excess$_{\text{Phyto-IW}}$ decreases with RH (from -210 ± 3 to -381 ± 19 per meg from 100 to 40 % RH) (fig. 1c, Table 1) according to the following equation (Eq.2):

$$^{17}$O-excess$_{\text{Phyto-IW}} = 4.4 \times \text{RH} - 554$$

Eq. 2

where $^{17}$O-excess$_{\text{Phyto-IW}}$ is expressed in per meg vs VSMOW and RH in %. $R^2$ is 0.94 and $p < 0.0001$ for the 95% confidence interval. This link between $^{17}$O-excess$_{\text{Phyto-IW}}$ and RH is mainly due to the leaf water $^{17}$O-excess dependency to RH since no particular trend is observed between $^{17}$O-excess$_{\text{Phyto-LW}}$ and RH and the raw values of $\theta_{\text{Phyto-LW}}$ appears constant, averaging 0.526 ± 0.004 (table 1). The coefficient, equivalent to 4.4 per meg / %, appears higher than the coefficient obtained for $^{17}$O-excess$_{\text{LW-IW}}$ vs RH (2.3 per meg / %). However, a Student’s t-test (relevant when the variance of two data sets are equal; Andrade and Estévez-Pérez, 2014), calculated on two data sets shows that for a 90% confidence interval, the slopes of the lines are not statistically different.

### 3.2 Natural samples

Values of $\delta^{18}$O$_{\text{Phyto}}$ and $^{17}$O-excess$_{\text{Phyto}}$ range respectively from 24.075 to 38.901 ‰ and from -140 to -290 per meg (table 2). The variations are in the same order of magnitude as for the growth chamber experiment. The estimates of $\delta^{18}$O$_{\text{Pre}}$ vary little along the sampled transect (from -4.458 to -3.220 ‰). No relationship is observed between $\delta^{18}$O$_{\text{Phyto}}$ or the $^{18}$O-enrichment of phytoliths relatively to precipitation ($\Delta^{18}$O$_{\text{Phyto-Pre}}$) and MAP, MAT or RH (fig. 3, table 2).

Although scattered, the $^{17}$O-excess$_{\text{Phyto}}$ values show a significant correlation with RH (fig. 4), regardless of which RH variable is taken into account. After excluding two outliers, the slopes of the correlation lines are 2.1 and 2.2 when RH and RH15 are taken into account, 3.4 when either RH-rd0>1 or RH15-rd0>1 are considered. The relationship obtained between $^{17}$O-excess$_{\text{Phyto}}$ and RH-rd0>1 is the closest to the one obtained between $^{17}$O-excess$_{\text{Phyto}}$ and RH in the growth chambers (fig. 4b). It can be expressed as follows (Eq.3):

$$^{17}$O-excess$_{\text{Phyto}} = 3.4 \times (\text{RH-rd0>1}) - 460$$

$(r^2 = 0.48; p < 0.001)$

Eq. 3

where $^{17}$O-excess$_{\text{Phyto}}$ is expressed in per meg vs VSMOW and RH in %.

The excluded outliers (Table 3) are RIM1 and C3L4. RIM1 presents a very low $^{17}$O-excess (-305 per meg) relatively to the $^{17}$O-excess of the samples with close RH-rd0>1, i.e. from 71 to 74 % (average of -237 ± 32 per meg for 82-78, 83-116 and 83-115). C3L4 is located next to C4L3 and
under similar averaged RH but presents a $^{17}$O-excess higher by 133 per meg. RIM1 and C3L4 show morphological patterns very similar to the other assemblages with the same range of RH. Thus, the discrepancies may lie either in the fact that local RH variations may not be reflected in RH averaged estimates for 10' ($\approx$ 185 km$^2$) or in the particularity of the isotope composition of the local soil water (see discussion below).

The phytolith index d/p ranges from 0.01 to 0.08 in savanna, from 0.14 to 0.49 in wooded savanna, from 0.76 to 1.58 in enclosed savanna and from 1.84 to 6.78 in humid forests (Table 2). This unambiguous increase of d/p with tree cover density is in agreement with previous calibrations performed for the West African area (Bremond et al., 2005b). Interestingly, under high RH conditions, humid forest and enclosed savanna that are characterized by a large range of d/p represent a small range of $^{17}$O-excess. Conversely, under lower RH conditions, savanna and wooded savanna that are characterized by a small range of d/p represent a large range of $^{17}$O-excess (fig. 5). This absence of relationship between $^{17}$O-excess and tree cover density is also mirrored in figure 4 where phytolith samples from different vegetation types (i.e. savanna vs wooded savanna or humid forests vs enclosed savanna), that have developed under the same RH conditions, have the same range of $^{17}$O-excess.

4 Discussion

4.1 Imprint of changes in atmospheric RH on the $^{17}$O-excess of leaf water

In the bulk leaf water, the trends observed between $\Delta^{18}_{LW IW}$ or $^{17}$O-excess$_{LW IW}$ and RH from 80 to 40 % are in agreement with an evaporative kinetic fractionation that increases when RH decreases, as expected from previous studies on the $^{18}$O$_{LW}$ or $^{17}$O-excess$_{LW}$ evolution (e.g. Cernusak et al., 2016; Landais et al., 2006; Li et al., 2017). The obtained averaged value of $\theta_{LW IW}$ (0.519 ± 0.002) is close to the value of $\theta_{diff}$ calculated for the diffusion of vapor in air (0.518; Barkan and Luz, 2007). As schematically described in Landais et al. (2016), $\lambda_{trans}$ (equivalent to $\theta_{LW IW}$) represents the interplay among three processes in the leaf boundary layer: 1) the equilibrium fractionation, which is only temperature-dependent (Majoube, 1971) and drives the isotope composition of leaf water along the equilibrium water line ($\theta_{equil} = 0.529$); 2) the diffusion transport leading to increasing kinetic fractionation with decreasing relative humidity along the diffusion line ($\theta_{diff} = 0.518$); 3) the isotopic exchange of leaf water with atmospheric water vapor, decreasing from turbulent to laminar and molecular leaf boundary layer vapor transport conditions (e.g. Buhay et al., 1996). In the case of the growth chamber experiment, the fact that $\theta_{LW IW}$ is close to $\theta_{diff}$ supports that the increasing diffusion of vapor in air when RH decreases or transpiration increases is the main process controlling the evolution of $^{17}$O-excess$_{LW}$. At high humidity (80-100% RH), the kinetic fractionation likely reaches its minimum as the diffusion process becomes limited.

The $\delta^{18}$O$_{LW}$ is commonly modelled as a function of the isotope composition of absorbed water, the isotope composition of water vapor, and RH (Craig and Gordon, 1965). The Craig and Gordon simple approach overestimates $\delta^{18}$O$_{LW}$ and different corrections have been proposed to take into account the diffusion of the evaporating water back to the leaf lamina and the advection of less
Almost a dozen temperature-dependant relationships have been empirically established between the $\delta^{18}O$ of quartz, sinters, cherts, diatoms or phytoliths and the $\delta^{18}O$ of their forming water. Polymerization of silica is supposed to occur in isotope equilibrium with the forming-water, and therefore, to be only governed by temperature and the isotope composition of the forming water. 

4.2 Imprint of changes in atmospheric RH on the $^{17}O$-excess of phytoliths

Finally, because wrong values of the isotope compositions of the water vapor may affect significantly the calculation of $\Delta^{18}LW-IW$, $^{17}O$-excess$_{LW-IW}$ and $\theta_{LW-SW}$, we call for vapor isotope measurements as a prerequisite to accurately model the leaf water triple oxygen isotope evolution with RH. However, overall, despite the uncertainties on the predicted evolution of $\theta_{LW-SW}$ with RH, the predicted value of $^{17}O$-excess$_{LW-IW}$ decreases when RH increases, which is also observed, as well as reflected in the triple isotope composition of phytoliths, as discussed below.
Although the obtained fractionation coefficients are close (from -0.2 to -0.4 ‰ °C⁻¹), the range of fractionation (Δ¹⁸Phyto-FW) is large (see synthesis in Alexandre et al., 2012). The Δ¹⁸Phyto-LW values obtained in the frame of the growth chamber experiment (ranging from 27.9 ± 7.2 to 32.3 ± 2.2‰) encompass the Δ¹⁸Phyto-FW of 31.1‰ calculated from the Dodd and Sharp (2010) relationship for 25°C. It is lower than the values of 36.4 and 36 %  at 25 °C, calculated from Sharp et al. (2016) and Alexandre et al. (2012). Whereas Alexandre et al. (2012) and Sharp et al. (2016) generally estimated the forming-water δ¹⁸O values, Dodd and Sharp (2010) measured the δ¹⁸O values of the water samples. The proximity of the obtained range of Δ¹⁸Phyto-LW values to the Δ¹⁸Phyto-FW calculated from Dodd and Sharp (2010) suggests that phytoliths formed in equilibrium with a water of isotope composition close to that of the bulk leaf water. This is additionally supported by the obtained averaged value of θPhyto-LW (0.526 ± 0.004), which is close to the 0°SiO2-water equilibrium value of 0.524 calculated for 25 °C from Sharp et al. (2016).

Evolution of the triple isotope composition of bulk leaf water and phytoliths can be illustrated by plotting δ¹⁷O vs δ¹⁸O, or ¹⁷O-excess vs δ¹⁸O (fig. 6) which is more appropriate to evidence small variations. Figure 6 shows that the leaf water evolved from the irrigation water pool, becomes increasingly subject to kinetic fractionation when RH decreased. This evolution follows a single leaf water line reflecting θLW-FW = 0.519 (Table 1). Then, if phytoliths polymerized from the bulk leaf waters, at 25°C, according to a constant equilibrium fractionation (Δ¹⁸Phyto-LW value close to 31.1‰, θPhyto-FW close to 0.524-0.526), the expected phytolith line in the ¹⁷O-excess vs δ¹⁸O diagram should be parallel to the leaf water line. Although the slope of the observed phytolith line appears slightly different from the expected one, a Student’s t-test calculated on the leaf water and phytolith data sets shows that for a 90% confidence interval, the slopes are not statistically different. This would support that phytoliths basically polymerized from the bulk leaf water and that evolution of their triple isotope composition is governed by that of the bulk leaf water, itself controlled by RH. However, when looking closer at the difference between δ¹⁸OPhyto and δ¹⁸OLW (fig. 6) or at Δ¹⁸OPhyto-LW (Table 1), for a given F. arundinacea sample, a significant increase can be observed when RH reaches 40 %. This indicates, on the contrary, a phytolith-forming water different from the bulk leaf water and more subject to kinetic fractionation. The Péclét effect, which is known to scale with transpiration (e.g. Barnard et al., 2007) can explain this discrepancy. Advection of less evaporated stem water may decrease δ¹⁸OLW and increase ¹⁷O-excess₈Phyto-LW relatively to δ¹⁸O and ¹⁷O-excess of the epidermal water prone to evaporation and from which phytoliths formed. This is however not shown by the ¹⁷O-excess₈Phyto-LW values, that are not significantly lower than that obtained at higher RH (except for one sample). At this point, the data scattering prevents further discussion but the discrepancy between Δ¹⁸OPhyto-LW and ¹⁷O-excess₈Phyto-LW evolutions at low RH and the possibility that the phytolith forming-water is different from the bulk leaf water must be investigated in future research developments.

With regard to the natural samples, whereas no relationship was found between δ¹⁸OPhyto and RH, a clear dependency of ¹⁷O-excess₈phyto to RH was shown, equivalent to 2.1 per meg / % when the
annual RH average was taken into account, or to 3.4 per meg / % when the average of the growing season (RH-rd0>1) was taken into account (fig. 4). These coefficients are in the range of those obtained for the growth chamber experiment for $^{17}O$-excess$_{\text{phyto}}$, $^{17}O$-excess$_{\text{s,W,TW}}$ and $^{17}O$-excess$_{\text{e Phyto-TW}}$. This consistency represents a major positive step in examining whether changes in atmospheric RH imprint the $^{17}O$-excess of natural phytolith assemblages in a predictable way. The data scattering ($r^2 = 0.48$ to 0.51, $p < 0.001$) however call for taking into account additional parameters beside RH that can contribute to changes in $^{17}O$-excess$_{\text{phyto}}$. One can expect the isotope composition of the soil water taken-up by the roots to impact $^{17}O$-excess$_{\text{phyto}}$. In tropical dry and humid areas, evaporative kinetic fractionation can lead to a $^{18}O$-enrichment of the soil water of several %o, in the first dm depth (e.g. Gaj et al., 2016; Liu et al., 2010). Spatial variability in the composition of the rainfall feeding the upper soil water may also intervene. However, the amount-weighted values of $^{18}O_{\text{prec}}$ along the sampled transect vary little (Table 2). With regard to $^{17}O$-excess, changes in soil water evaporation rather than the small variations expected for $^{17}O$-excess$_{\text{pre}}$ (Landais et al., 2010b; Li et al., 2015) should impact the evolution of $^{17}O$-excess$_{\text{phyto}}$ although, here, the lack of measurements only allow for speculation.

The source vegetation of the phytolith assemblages may also bring some noise to the relationship between $^{17}O$-excess$_{\text{phyto}}$ and RH. The Globular granulate phytoliths that are assumed to come from the non-transpiring secondary xylem of the wood should present an isotope signature closer to that of the soil water, or less impacted by kinetic fractionation than the grass phytoliths. However, for a given range of RH, samples with significant representations of both phytolith categories (i.e. wooded savanna and enclosed savanna with d/p from 0.1 to 1.6) present $^{17}O$-excess values close to the values obtained by samples with very low or very high d/p (figs. 4 and 5). To further assess the significance of the Globular granulate isotope signature, we calculated $^{18}O_{\text{phyto,FW}}$ values (Table 2) using the Dodd and Sharp (2010) fractionation factor and compared it to the precipitation-weighted $^{18}O_{\text{pre,rd0>1}}$ average. For the humid forest assemblages, $^{18}O_{\text{phyto,FW}}$ values are higher than $^{18}O_{\text{pre,rd0>1}}$ by 5 ± 1.4 %. This difference is larger than the range of $^{18}O$-enrichment observed for the tenths upper cm depth of soil water under tropical humid forests (2-3‰; Liu et al., 2008; Stahl et al., 2013), suggesting that evaporative isotope signatures of both soils and leaf water imprinted the Globular granulate phytolith type. Complementary examination of the isotope signature of phytolith assemblages from forests growing under different RH conditions (i.e. dry forests, humid forests, rainforests), as well as further investigation of the anatomical origin of the Globular granulate phytolith type are now required to further discuss the meaning of the $^{17}O$-excess signal brought by tropical forest phytolith assemblages.

Biases due to the calibration methodology may also be responsible for the data scattering. Imperfect adequation between the space scales recorded by the soil top phytolith assemblages and the RH variables may come into play. Phytolith assemblages represent a mixture of local and wind-transported phytoliths. In the open saharian, sahelian and soudanian zones of West Africa the winter low altitude north-easterly trade winds may transport phytoliths southward, reducing differences between assemblages from different biogeographic zones and increasing differences among assemblages of a given biogeographic zone (Bremond et al., 2005b). Additional samples
from other geographic zones are thus needed to increase the robustness of the relationship. With regard to the recorded time scales, phytolith assemblages likely record several decades of phytolith production in agreement with the CRU RH 30 years averages.

5 Conclusion

The present combination of growth chamber and in situ transect calibrations lay the groundwork for further examination of the robustness of the \( ^{17}\text{O}-\text{excess}_{\text{phyto}} \) as a proxy of changes in RH. The growth chamber experiment demonstrated that change in RH imprint the \( ^{17}\text{O}-\text{excess}_{\text{phyto-IW}} \) (by 4.4 per meg / %) through its imprint on \( ^{17}\text{O}-\text{excess}_{\text{LW-IW}} \). As the isotope composition of the irrigation water was stable, and transpiration likely reached a steady state, the positive correlation between \( ^{17}\text{O}-\text{excess}_{\text{LW}} \) and RH was only governed by the kinetic fractionation occurring in the leaf epidermis water subject to evaporation, as supported by the value of \( \theta_{\text{LW-IW}} \) of 0.519, close to \( \theta_{\text{diff}} \).

In order to model the triple oxygen isotope fractionation in play at the soil/plant/atmosphere interface we require direct and continuous measurements of the triple isotope composition of water vapor. Such measurements should develop in the near future through the use of isotope ratio infrared analyzers (e.g. Berkelhammer et al., 2013; Schmidt et al., 2010). We also suggest to constrain as much as possible the isotope composition of the soil water taken up by the roots. Stem water is usually used as an analogue of soil water when modelling \( \delta^{17}\text{O}_{\text{LW}} \) and \( \delta^{18}\text{O}_{\text{LW}} \) (Landais et al., 2006; Li et al., 2017). However, in the stem, water in the phloem that is bidirectional (moves up and down the plant’s stem) receives the contribution of evaporating leaf water, whereas water in the xylem that is unidirectional (moves up the plant’s stem) is not influenced by leaf evaporation. Consequently one may expect the isotope composition of stem water to be slightly different than that of soil water (Berkelhammer et al., 2013; Treydte et al., 2014).

When plotting \( ^{17}\text{O}-\text{excess}_{\text{phyto}} \) vs RH, the samples collected along the West and Central African relative humidity transect lay close to the growth chamber \( ^{17}\text{O}-\text{excess}_{\text{phyto-IW}} \) line and define a correlation coefficient ranging from 2.1 to 3.4 per meg / % (depending on the RH variable taken into account). This supports that RH is an important control of \( ^{17}\text{O}-\text{excess}_{\text{phyto}} \) in natural environment, even if phytolith assemblages come from different vegetation types. However, other parameters such as changes in the triple isotope composition of the soil water or imperfect adequation between the space scales recorded by the soil top phytolith assemblages and the RH variables may come into play and explain the scattering of \( ^{17}\text{O}-\text{excess}_{\text{phyto}} \). Assessment of these parameters through additional growth chambers experiments and field campaigns will bring us closer to an accurate proxy of changes in relative humidity.

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Table 1. Growth chamber experiment: experimental set-up, phytolith content and morphological characteristics, isotope enrichments ($\Delta_{A-B} = (\delta_a - \delta_b) / (\delta_a / 1000 + 1)$), associated $^{17}$O-excess, $^{17}$O-excess = $\ln (\Delta_{17} + 1) - 0.528 \times \ln (\Delta_{18} + 1)$ and $\theta = \ln (\Delta_{17_{A-B}} + 1) / \ln (\Delta_{18_{A-B}} + 1)$ values of phytoliths compared to either leaf water or irrigation water and of leaf water compared to irrigation water. $\overline{Av}$: average; $n$: number of replicates; $SD$: standard deviation calculated on the replicates; n.v.: no value. Transp. (l/day), Conc. (% d.w.) and LC (%) stands for transpiration expressed in liter/day, phytolith concentration expressed in % of the dry weight and long cell abundance in the phytolith morphological assemblage expressed in % of counted phytoliths with taxonomic significance, respectively. $^{17}$O-excess = $\ln (\delta^{17}O + 1) - 0.528 \times \ln (\delta^{18}O + 1)$. 

Table 1. Growth chamber experiment: experimental set-up, phytolith content and morphological characteristics, isotope enrichments ($\Delta_{A-B} = (\delta_a - \delta_b) / (\delta_a / 1000 + 1)$), associated $^{17}$O-excess, $^{17}$O-excess = $\ln (\Delta_{17} + 1) - 0.528 \times \ln (\Delta_{18} + 1)$ and $\theta = \ln (\Delta_{17_{A-B}} + 1) / \ln (\Delta_{18_{A-B}} + 1)$ values of phytoliths compared to either leaf water or irrigation water and of leaf water compared to irrigation water. $\overline{Av}$: average; $n$: number of replicates; $SD$: standard deviation calculated on the replicates; n.v.: no value. Transp. (l/day), Conc. (% d.w.) and LC (%) stands for transpiration expressed in liter/day, phytolith concentration expressed in % of the dry weight and long cell abundance in the phytolith morphological assemblage expressed in % of counted phytoliths with taxonomic significance, respectively. $^{17}$O-excess = $\ln (\delta^{17}O + 1) - 0.528 \times \ln (\delta^{18}O + 1)$. 

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(a) Calculated on the raw values
(b) Calculated on the raw values without taking into account sample P1-40-29-04-16.
Table 2. Natural West and Central African samples: coordinates, climatic parameters, d/p, $\delta^{18}O$, $\delta^{13}O$, $\delta^{17}O$, $\delta^{15}O$-excess for the phytolith assemblages. Average and standard deviation (SD) are given for replicates. MAP: Mean Annual Precipitation; MAT: Mean Annual Temperature; RH: mean annual relative humidity; RH15: RH at 15:00 H UTC; RH-rd0=1: relative humidity average for months with days with precipitation higher than 0.1 mm; RH15-rd0=1: RHrd0≥1 at 15:00 H UTC. See text for data source and calculation.

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Figure captions

Figure 1. Growth chamber experiment: a) $^{17}$O-excess vs relative humidity (RH) of irrigation water (IW), soil water (SW), leaf water (LW) and phytolith (Phyto). Error bars show standard deviation (SD) on the replicates. They are smaller than the symbol when not shown. b) $^{17}$O-enrichment from irrigation water to leaf water ($^{18}$D$_{LW-IW}$), from irrigation water to phytolith ($^{18}$D$_{Phyto-IW}$) and from leaf water to phytolith ($^{18}$D$_{Phyto-LW}$). c) $^{17}$O-excess associated with the enrichment from irrigation water to leaf water ($^{17}$O-excess$e_{LW-IW}$), from irrigation water to phytolith ($^{17}$O-excess$e_{Phyto-IW}$), and from leaf water to phytolith ($^{17}$O-excess$e_{Phyto-LW}$).

Figure 2. Growth chamber experiment: phytolith types extracted from Festuca arundinacea and observed in natural light microscopy: epidermal long cell (LC), epidermal short cell (SC).

Figure 3. Natural West and Central African transect: $\delta^{18}$O of phytoliths ($\delta^{18}$O$_{Phyto}$) vs relative humidity RH-rd0>1 (see fig. 4 for explanation). Error bars show standard deviation (SD) on the replicates. When not shown, they are smaller than the symbol. For comparison, the $\Delta^{18}$O$_{Phyto-IW}$ vs RH line is plotted for comparison.

Figure 4. Natural West and Central African transect: $^{17}$O-excess vs relative humidity (RH) of phytolith assemblages from soil tops collected under savanna, wooded savanna, humid forest and enclosed savanna along a humidity gradient (Table 1). The growth chamber $^{17}$O–excess$_{Phyto-IW}$ vs RH correlation line is displayed for comparison. a) RH-Av: yearly average of monthly means; b) RH-rd0>1: yearly average of monthly means for months with at least one day with precipitation higher than 0.1mm; c) RH15: RH at 15:00 H UTC; d) RH15-rd0>1: RH-rd0>1 at 15:00 H UTC.

Figure 5. Natural West and Central African transect: $^{17}$O-excess of phytoliths ($^{17}$O-excess$_{Phyto}$) vs d/p.

Figure 6. Growth chamber experiment: $^{17}$O-excess vs $\delta^{18}$O of irrigation water (IW), soil water (SW), leaf water (LW) and phytolith (Phyto). Error bars show standard deviation (SD) on the replicates. They are smaller than the symbol when not shown. The observed bulk leaf water line reflecting $\theta_{LW-IW} = 0.519$, the expected phytolith line in agreement with phytoliths polymerizing from the bulk leaf water, and the observed phytolith line are displayed. See text for explanation.
Figure 1

**Figure 1**

(a) 

\[ y = 2.4 - 239 \]

\[ R^2 = 0.78 \]

\[ y = 4.1x - 521 \]

\[ R^2 = 0.88 \]

(b) 

\[ y = 0.320x + 62.391 \]

\[ R^2 = 0.92 \]

\[ y = -0.098x + 35.998 \]

\[ R^2 = 0.40 \]

\[ y = -0.204x + 24.868 \]

\[ R^2 = 0.67 \]

(c) 

\[ y = 2.3x - 258 \]

\[ R^2 = 0.72 \]

\[ y = 4.4x - 554 \]

\[ R^2 = 0.94 \]
Figure 2
Figure 3
Figure 4

(a) \( y = 2.1x - 342 \), \( r^2 = 0.51 \)

(b) \( y = 3.4x - 460 \), \( r^2 = 0.48 \)

(c) \( y = 2.2x - 321 \), \( r^2 = 0.50 \)

(d) \( y = 3.4x - 411 \), \( r^2 = 0.46 \)

- Savanna
- Humid forest
- Growth chamber
- \( ^{17}O\) excess per mg vs VSMOW
- \( ^{18}O\) excess per mg vs VSMOW
- Wooded savanna
- Enclosed savanna
- Outlier
Figure 5
Figure 6

[Graph showing water isotopes and phytooliths]